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Potency of Probiotics on HDL, LDL, Cholesterol and Total Protein of Egg's Quail (*Coturnix coturnix japonica*)

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ABSTRACT

The purpose of this research was to know the potency of probiotic supplementation to content of HDL, LDL, Cholesterol and total protein of egg's quail *Coturnix coturnix japonica*. This research consist of five treatments, each treatment consisted of four replications and each replication consisted by ten quail. The treatment were T0: control (without probiotic), T1: 0.05 gram probiotic/ kg feed, T2: 0.1 gram probiotic/kg feed, T3:0.025 gram probiotic/L drinking water and T4: 0.05 gram probiotic/L drinking water. The probiotic consisted of combine *Lactobacillus casei* and *Lactobacillus rhamnosus* of 1.2×10^8 CFU/gram. The results showed that the probiotic supplementation both in feed and water gave a significant impact to reduce of Low Density Lipoprotein (LDL) and cholesterol, improve of High Density Lipoprotein (HDL) level. Moreover, the quails feed with free antibiotic feed with free antibiotic feed + 0.025 gram probiotic/L drinking water and 0.1 gram probiotic/kg feed could reduce ($P < 0.05$) egg cholesterol, LDL and could improve HDL and the total proteins. It can be concluded that using probiotic contain *Lactobacillus casei* and *Lactobacillus rhamnosus* could improve egg quality by reduced cholesterol, LDL and increase HDL and total protein of egg's quail *Coturnix coturnix japonica*.

KEYWORDS: Probiotic, HDL, LDL, Cholesterol, total protein

INTRODUCTION

Quail has advantageous attributes such as small body size, rapid growth rate and early sexual maturity [1]. Quail also has cheaper production cost, and don't need a large land, easy to handle by human. Therefore they used for commercial rearing, egg and meat production under intensive management [2].

Probiotics are defined as beneficial live microorganisms, when administered in adequate amount may support a beneficial health on people with specific illnesses [3]. Probiotics, including live yeasts and bacteria, as feed additive or supplements and known give beneficial effect to the host by improving intestinal microbial balance, producing several substances that act as antimicrobial, stimulating the immune response, increase the intestinal microflora balance, modulating the immune response, and lowering cholesterol levels, enhance nutrient bioavailability, carcass yield and quality of Japanese quail [4, 5].

Lactobacillus sp. one of the isolates of bacteria that has a potential as a probiotic from the group of lactic acid bacteria. Lactic acid bacteria are Gram-positive, coccus or rod, non spore, acid tolerant, catalase negative, facultative an aerob and the major end product was lactic acid. The use of lactic acid bacteria provides benefits for animals and humans against pathogenic bacteria. Prolonged and continuous use of antibiotics in animal feed may result in antibiotic resistance. Probiotics such as lactic acid bacteria can be used as a substitute for antibiotic growth promoters that have a positive impact on livestock growth and disease prevention. Several studies have shown that the use of probiotics can decrease triglyceride and cholesterol content in serum and egg yolks [6-9]. The purpose of this research to evaluate the effect of probiotics supplementation containing *Lactobacillus casei* and *Lactobacillus rhamnosus* as alternative Antibiotic Growth Promoter to HDL, LDL, cholesterol and total protein of egg's *Coturnix coturnix japonica*.

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MATERIALS AND METHODS

About 200 of 14weekold quails were randomized into five treatments with four repetitions, each replication consisted of ten quails, with this following treatment: T0: control (without probiotic), T1: 0.05 gram probiotic/ kg feed, T2: 0.1 gram probiotic/kg feed, T3: 0.025 gram probiotic/L drinking water and T4: 0.05 gram probiotic/L drinking water. Egg's quail were collected every day. The data collection of this research by taking five eggs randomly on each repetition in the last week of treatment.

a. **Cholesterol**, samples and reagents are prepared at roomtemperature after that pipette into tubes as below:

Tubes	Blank	Sample	CAL. Standard
R1. Monoreagent	1.0 mL	1.0 mL	1.0 mL
Sample	-	10 µL	-
CAL. Standard	-	-	10 µL

Mixingsamples and reagents until homogen and incubate 10 minutes or 5 minutes at room temperature (37°C). After incubate, record the absorbance (A) of the standard and samples at 500 nm against the reagent blank.

The color will be stable for 30 minutes and protected it from sunlight.

Calculations of total cholesterol was total cholesterol (mg/dL) = A sample/A standard × C standard

1 using samples with concentrations higher than 600 mg/dL, it should be diluted with saline to be 1:2 and then assayed again. Multiply the result by 2.

If results are to be expressed as SI units apply: mmol/L = mg/dL × 0.0259

Cholesterol assay procedure,

Temperature at 25°C- 37°C, Optical path 1 cm, 500 nm and Hg 546 nm

Measurement Against reagent blank

	Sample	Standard
Supernatant	100 µL	-
Standard	-	100 µL
Reagent	1000 µL	1000 µL

Calculation of cholesterol

Cholesterol in supernatant (mg/dL) = Δ E sample/ Δ E standard × concentration standard (mg/dL)

The concentration standard is the concentration of the total cholesterol in the cholesterol standard solution.

- I. **HDL assay procedure:** samples and reagents prepare in roomtemperature and pipette into centrifuge tubes 0.2 mL standard or sample and 0.4 mL precipitating reagent with ratio sample/reagent = 1/2 and dilution factor = 3. Vortex and allow to stand at room's temperature for 10 minutes after that centrifuge at 4000 rpm for 10 minutes, or at 12000 rpm for 2 minutes. Separate the clear supernatant within 2 hours. If supernatant triglycerides (>350 g/dL) the sample should be diluted with saline (1:2) and then assayed again. Multiply the result by 2.

II.

- II. Colorimetry : the cholesterol standard (50 mg/dL) and the cholesterol MR Monoreagent of the kit prepare in room's temperature and pipette into tubes as below:

TUBES	Blank	Standard	Sample
		1 Supernatant	Supernatant
Monoreagent	1.0 mL	1.0 mL	1.0 mL
Supernatant	-	-	50 µL
Standard	-	50 µL	-

Mixing and let the tubes at room temperature for 10 minutes or at 37°C for 5 minutes and read the absorbance (A) of the supernatant and the standard at 500 nm against the reagent blank. The color will be stable for 30 minutes and protected it from sunlight.

Calculations of HDL:

HDL - cholesterol (mg/dL) = A supernatant/A standard × C standard

The results are expressed as SI units: mmol/L = mg/dL × 0.0259

b. LDL

Assay procedure

Precipitation

Sample 100µL and precipitating reagent 1000 µL

Mix and incubate at room temperature for 15 minutes, then centrifuge for 20 minutes at 2500 g. Within one hour after centrifugation, transfer 100 µL of the clear supernatant to the reaction solution for the determination of cholesterol.

The cholesterol standard has to be diluted 1 + 10 with NaCL (9 g/L). After dilution the standard is treated like the supernatant.

c. Protein assay procedure

Pipette into tubes:

TUBES	Blank	Sample	3 L Standard
R1.Biuret	1.0 mL	1.0 mL	1.0 mL
Sample	-	20 μ L	-
CAL. Standard	-	-	20 μ L

Mixed and incubated the tubes for 10 minutes at 37°C and read the absorbance (A) of the samples and the standard at λ 540 nm against the reagent blank. The color will be stable for at least 1 hour

Calculations of total protein:

Total protein (g/dL) = A sample/A standard \times C standard

Samples with concentrations higher than 12 g/ dL should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

If results are to be expressed as SI units apply: g/ dL \times 10= g/L

Statistical Analysis

Data analysis use Analysis of Variant (ANOVA). If the result show different or significantly different then continue with Duncan Multiple Range Test[10]. Statistical analysis using SPSS for Windows 21.0.

RESULTS

Cholesterol, HDL, LDL of egg's quail

The results of *Analysis of Variance* (ANOVA) showed that the addition of probiotics through feed and drinking water have an effect on egg's HDL, LDL and cholesterol ($p < 0.05$) compare with control (Table 1).

Table 1. HDL, LDL, and Cholesterol Levels of egg quail in 4 week of treatment

Treatment	HDL(mg/g)	LDL(mg/g)	Cholesterol(mg/g)
T0	4.15 ^b \pm 0.01	1.38 ^b \pm 0.01	19.02 ^d \pm 0.02
T1	4.68 ^d \pm 0.02	1.49 ^c \pm 0.01	16.74 ^b \pm 0.01
T2	7.15 ^e \pm 0.02	1.27 ^a \pm 0.01	22.97 ^e \pm 0.02
T3	3.88 ^a \pm 0.01	1.74 ^d \pm 0.02	10.41 ^a \pm 0.01
T4	4.64 ^c \pm 0.02	1.38 ^b \pm 0.01	17.23 ^c \pm 0.02

Total protein of egg's quail

The results of *Analysis of Variance* (ANOVA) showed that the addition of probiotics through feed and drinking water have an effect on egg's total protein content ($p < 0.05$) compare with control (Table 2).

Table 2. Total Protein of egg quail in 4 week of treatment

Treatment	Total Protein(mg/g)
T0	136.43 ^a \pm 0.02
T1	215.48 ^e \pm 0.01
T2	168.81 ^b \pm 0.02
T3	212.62 ^d \pm 0.02
T4	194.05 ^c \pm 0.02

DISCUSSION

The results of the quail egg cholesterol showed a differences compared to the controls (Table 1). In T3 treatment (0.025 gram probiotic/L drinking water) showed the highest significantly different effect which means T3 (10.41 mg/g) has the lowest cholesterol content compared with T0 (19.02 mg/g) and the others treatment groups (Table 1). Treatment T3 was the best result that can reduce the yolk cholesterol. To know the most influence treatment continued with Duncan's Multiple Range Test. The lowering of cholesterol levels in the egg yolk from the treatment caused by the amount of probiotics administration through drinking water which more effective to produce the amount of microbes in the digestive tract. This result in line with the research[11] that probiotics *Lactobacillus* Phussupplementation with treatment 0, 0.2%, 0.4% and 0.6% consisted by 2.56×10^8 CFU showed significantly different in egg cholesterol. Increasing the number of microbes inhibit Hydroxi Metyl Glutaryl-KoA reductase (HMG-KoA reductase) enzyme work which plays a role in the formation of mevalonate in the process of cholesterol synthesis so can't change into cholesterol.

The research on improving egg characteristics is essential, not only to produce better quality eggs but also to provide food safety in a balanced diet for poultry industries [12]. Cholesterol is important for health because it is used as a constituent of hormones and for the production of bile acids [13], but excessive cholesterol consumption will endanger the health because it can cause atherosclerosis (blockage of arteries).

The results of Analysis of Variance (ANOVA) in HDL levels of quail eggs showed significant difference ($P < 0.05$) among treatments. The results of analysis by Duncan's Multiple Range test showed differences in HDL levels among egg yolk T0 with T1, T2, T3 and T4. The highest HDL showed in 0.1 gram probiotic/kg feed (T2) treatment. This is in line with the study [14] that the fermented synbiotics supplementation affect to the HDL enhancement or good fats in laying hens. The fermentation synbiotics supplementation to 30% level in feed can raise blood serum HDL in laying hens. Supplementation of protexin into the laying hens diet has been shown to reduce egg yolk and serum cholesterol level. The mean of serum cholesterol level in the study was decrease from 188.50 mg/dL in the control group to be 186.35 mg/dL in the probiotic supplementation group. It is possible that microorganisms in the probiotics can assimilate the cholesterol in the tractus digestivus for cell metabolism, thus reducing the total absorption [15]. Another reason to reduce the cholesterol in host which fed by probiotics, suggested by [16] that probiotics could inhibit enzyme hydroxymethyl-glutaryl-CoA enzyme A, which is involved in the gastrointestinal tract. Another mechanism through probiotics can cause hypercholesterolemia action through bile acids (deoxycholic bile acids and cholic) which produced by hepatocytes and conjugated with taurine and glycine amino acid. In the small intestine, this acid absorbed and directed to the liver, and lower bile acid recycling will eventually lower serum cholesterol level. Probiotics can affect the binding of cholesterol and the inhibition of micelle formation combined with the fermentation effect on short chain fatty acid (SCFA) production is a proposed mechanism for explaining potential cholesterol effects [17]. Probiotics may change the enzyme, which is related in the process.

The enhancement of HDL levels usually followed by reduction levels of LDL and cholesterol. The results of LDL levels showed significant differences ($P < 0.05$) among treatments. The lowest LDL (1.27 mg/g) and high HDL (7.15 mg/g) showed by 0.1 gram probiotic/kg feed (T2) treatment. The reduction in LDL caused by the balances between HDL and LDL levels. The statement in line with [18] which states that the feed supplement can increase HDL levels and directly affect the reduction in LDL levels. LDL that has been formed in the liver will be sent to the blood; the over production of LDL will be bound by Lecithin - Cholesterol Acyl Transferase (LCAT) enzyme in the process of Reverse Cholesterol Transport (RCT). LCAT has a function to bind lipoproteins or free fat in the blood plasma and send it to the liver. HDL as a lipoprotein that plays a role in the RCT process will increase the transport of cholesterol from the tissues to be returned to the liver and excreted through bile [19].

Total Protein

Based on the results of statistical analysis (Table 2) showed that 6th treatment had significantly different effect ($P < 0.05$) on quail egg yolk protein level. The result showed that the addition of probiotics in feed and drink can increase protein levels. The high average of egg yolk protein showed in T1 (215.48), T3 (212.62), T1 (207.62), T4 (194.05) and T2 (168.81 mg/g). The lowest total protein content of egg showed in control treatment (T0) without addition of probiotic. Protein content in feed affects the protein composition in eggs. Protein in feed mostly used for egg production, only a small portion for basic living; higher production level then need higher protein [20]. The addition of probiotics in feed can increase microbial activity, enzyme activity and protein digestibility as well as metabolic energy of feed in the quail's digestive tract, so can accelerate the rate of feed movement, so the absorption of feed substances become larger and impact the enhancement of feed efficiency and quail production [21]. So that the protein content in probiotics can affect the protein content in quail eggs.

CONCLUSION

The results showed that probiotics 0.1 gram /kg feed and 0.025 gram /L drinking water could reduce cholesterol level, LDL level, increase HDL level and total protein of egg's quail *Coturnix coturnix japonica*.

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